

LIS009089583B2

# (12) United States Patent

Sun et al.

(54) PHARMACEUTICAL COMPOSITION FOR STRENGTHENING SPLEEN AND STOMACH AND PREPARATION METHOD THEREFOR

(75) Inventors: **Dejun Sun**, Jilin (CN); **Jinlong Yin**,

Jilin (CN); Miaonan Sun, Jilin (CN); Yizhuo Zhao, Jilin (CN); Chunsheng Guo, Jilin (CN); Yanhui Gao, Jilin

(CN); Xue Li, Jilin (CN)

(73) Assignee: Jilin Zixin Pharmaceutical Research

Institution LLC., Changchun, Jilin (CN)

(\*) Notice: Subject to any disclaimer, the term of this

patent is extended or adjusted under 35

U.S.C. 154(b) by 0 days.

(21) Appl. No.: 13/980,878

(22) PCT Filed: Jul. 15, 2011

(86) PCT No.: PCT/CN2011/077176

§ 371 (c)(1),

(2), (4) Date: Jul. 20, 2013

(87) PCT Pub. No.: WO2012/097578

PCT Pub. Date: Jul. 26, 2012

(65) **Prior Publication Data** 

US 2013/0295079 A1 Nov. 7, 2013

# (30) Foreign Application Priority Data

Jan. 21, 2011 (CN) ...... 2011 1 0023836

(51)	Int. Cl.	
	A61K 35/00	(2006.01)
	A61K 35/66	(2015.01)
	A61K 36/076	(2006.01)
	A61K 36/258	(2006.01)
	A61K 36/284	(2006.01)
	A61K 36/484	(2006.01)
	A61K 36/62	(2006.01)
	A61K 36/734	(2006.01)
	A61K 36/752	(2006.01)
	A61K 36/884	(2006.01)
	A61K 36/8945	(2006.01)
	A61K 36/8998	(2006.01)
	A61K 35/745	(2015.01)
	A61K 35/747	(2015.01)

(52) U.S. Cl.

 (10) **Patent No.:** 

US 9,089,583 B2

(45) **Date of Patent:** 

Jul. 28, 2015

36/076 (2013.01); A61K 36/258 (2013.01); A61K 36/284 (2013.01); A61K 36/484 (2013.01); A61K 36/62 (2013.01); A61K 36/734 (2013.01); A61K 36/752 (2013.01); A61K 36/884 (2013.01); A61K 36/8945 (2013.01); A61K 36/8998 (2013.01)

# (58) Field of Classification Search

None

See application file for complete search history.

#### (56) References Cited

#### FOREIGN PATENT DOCUMENTS

CN 101091778 A \* 12/2007 EP 1169925 A1 \* 1/2002

#### OTHER PUBLICATIONS

Yan, Shi Lin and Li, Zheng-Hau, Pathomechanisms of the Spleen, translated by Sabine Wilms, edited by Nigel Wiseman, Paradigm Publications, Taos, New Mexico, 2009.\*

Yoon et al. "Fermentation of beet juice by beneficial lactic acid bacteria", Lebensmittel-Wissenschaft & Technologie 38: 73-75, 2005.\*

Frauenfelder "Making sauerkraut is easy", Boing Boing website, available online 2009.\*

Kotzamanidis et al. "Optimization of lactic acid production from beet molasses by *Lactobacillus delbrueckii* NCIMB 8130", World Journal of Microbiology & Biotechnology 18: 441-8, 2002.\*

Xu, CN 101091778 A, GPSN translation.\*

Effect of bifidobacterium fermented mixed fruit and vegetable juice on immunological function of mice.

Primary Examiner — Ruth Davis Assistant Examiner — Emily Cordas

#### (57) ABSTRACT

A pharmaceutical composition for strengthening spleen and stomach and a preparation method therefor. The method includes the step of mixing four substances listed below to obtain a mixture, wherein the mixture is the pharmaceutical composition for strengthening spleen and stomach, and the four substances are (A) ginseng ethanol extract, (B) volatile oil of largehead atractylodes rhizome and tangerine peels, (C) aqueous extract of Indian bread, yamaimo, lotus seeds, tangerine peels, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome and rhizoma alismatis; (D) fermentation broth of fruits and vegetables.

# 2 Claims, No Drawings

<sup>\*</sup> cited by examiner

### PHARMACEUTICAL COMPOSITION FOR STRENGTHENING SPLEEN AND STOMACH AND PREPARATION METHOD THEREFOR

# CROSS REFERENCE OF RELATED APPLICATION

This is a U.S. National Stage under 35 U.S.C. 371 of the International Application PCT/CN2011/077176, filed Jul. 15, 2011, which claims priority under 35 U.S.C. 119(a-d) to CN 10 20110023836.X, filed Jan. 21, 2011.

#### BACKGROUND OF THE PRESENT INVENTION

#### 1. Field of Invention

The present invention relates to a pharmaceutical composition for strengthening spleen and stomach and a preparation method therefor.

#### 2. Description of Related Arts

Dyspepsia is a clinical syndrome, which is a disease caused 20 by gastric dynamic dysfunction. Dyspepsia also includes gastroparesis and gastroesophageal reflux disease caused by bad peristole. Dyspepsia could be mainly divided into functional dyspepsia and organic dyspepsia. The symptoms are discomfort or pain in the upper abdominal intermittent, fullness, 25 heartburn (reflux), belching, etc. The patient usually does not want to take food or takes food as few as possible because of discomforts such as sense of suppression in the chest, early full feeling, and abdominal distension. The patient could not fall asleep easily at night, and usually has nightmares after 30 falling asleep. In the medical inspection of the hospital, except that mild gastritis could be detected under the gastroscope, other abnormal performances could not be detected through other inspections, such as Bultrasound, radiography, and blood biochemical test.

Ginseng is the root of the Araliaceae plant ginseng. More than 30 kinds of panaxoside and polysaccharide could be separated from the ginseng.

Largehead atractylodes rhizome is the dry rhizome of the Asteraceae plant Atractylodes macrocephala Koidz. The 40 largehead atractylodes rhizome is bitter, sweet, warm, and benefit for spleen and stomach. The largehead atractylodes rhizome could strengthen the spleen, tonify vitality, eliminating dampness, promote dieresis, suppress sweating, and prevent abortion. The largehead atractylodes rhizome is indicated for asthenia of the spleen, deficiency of food, abdominal distention, diarrhea, phlegm retention, giddiness, palpitation, edema, apontaneous perspiration, and fetal irritability.

Indian bread is a fungus parasitizing on the root of pine trees. The Indian bread is sweet in taste, bland in flavour, and 50 neutral in nature. The Indian bread is benefit for heart, lung, spleen, and kidney. The Indian bread has functions of promoting dieresis, excreting dampness, strengthening spleen and stomach, relieving mental stress.

Yamaimo is the root of yamaimo plant. The yamaimo is 55 sweet in taste, warm, neutral in nature. The yamaimo is benefit for lung, spleen, and kidney. The yamaimo is indicated for weakness of the spleen and the stomach, listlessness, myasthenia, inappetence, prologed diarrhea and dysentery, deficiency of lung-vitality, dryness of the lung, phlegm dyspnea, 60 cough, deficiency of kidney, soreness of the waist and knees, weakness of the lower extremity, diabetes, pollakiuria, emission and premature ejaculation, leukorrhagia, blennorrhea, red swollen skin, adiposis, etc.

Lotus seed is the dry mature seed of the lotus, which is a 65 Nymphaeaceae aquatic herbaceous plant. The lotus seed is sweet in taste, cool in nature, and avirulent. The lotus seed is

2

benefit for the spleen, the kidney, and the heart. The lotus seed has the functions of clearing away the heart-fire, activating the spleen-vitality, strengthening the spleen, anti-diarrhea, nourishing the heart, tranquilizing the mind, improving the eyesight, strengthening the vitality of the spleen and the stomach, cultivating the mentality, strengthening spleen and stomach, stopping emission, strengthening the kidney, treating spontaneous emission and leukorrhagia, and nourishing the archaeus. The lotus seed is indicated for hypochondriac discomfort, insomnia, weakness of the spleen, long-lasting diarrhea, sticky loose stools, protracted dysentery, lumbago, apontaneous emission of males, and leuhorrhagia with reddish discharge of females. The lotus seed could also prevent premature birth, abortion, and loins soreness of the pregnant woman.

Hawthorn fruit is the dry mature fruit of Shan Lihong or hawthorn, which is a Rosaceae Maloideae plant. The hawthom fruit is sour and sweet in taste, slightly warm in nature. The hawthom fruit is benefit for the spleen, stomach, and liver. The hawthom fruit has functions of improving appetite, promoting digestion, relieving dyspepsia, promoting blood circulation, desslpating blood stasis, reducing phlegm, and promoting the circulation of vitality.

Germinated barley is the dried germinant mature fruit of the gramineae plant *Hordeum vulare L*. The germinated barley is sweet in taste, neutral in nature. The germinated barley is benefit for spleen and stomach. The germinated barley has functions of promoting the circulation of vitality and digestion, strengthening the spleen, improving appetite, suppression of lactation, and relieving flatulence. The germinated barley is indicated for dyspepsia, abdominal swelling and pain, weakness of the spleen, deficiency of food, galactostasis, distending pain in the breast, and delectation of woman.

Rizoma alismatis is the tuber of Alismataceae plant Rizoma alismatis. The Rizoma alismatis is sweet in taste, bland in flavor, cold in nature. The Rizoma alismatis is benefit for the kidney and bladder. The Rizoma alismatis has the functions of promoting dieresis, excreting dampness, letting off heat, and treating stranguria. The Rizoma alismatis is indicated for difficult urination, heat gonorrhea, painful and diffecult hematuria, edema, turgor, diarrhea, dizziness due to phlegmretention, and apontaneous emission.

Tangerine peel is the peel of the Rutaceae plant tangerine. The tangerine peel is acrid and bitter in taste, and warm in nature. The tangerine peel is benefit for the spleen and the lung. The tangerine peel has functions of regulating the flow of vitality and the middle warmer, eliminating dampness, and reducing phlegm. The tangerine peel is indicated for stagnation of vitality in the spleen and stomach, distension in the abdomen, vomiting, or chest distress, anorexia, and soft stool due to stagnation of turbid dampness.

Liquorice root is the root and rhizome of the dicotyledon leguminosae liquorice. The liquorice root is neutral in nature, and sweet in taste. The liquorice root is benefit for twelve regular channels. The liquorice root has pharmacological actions of detoxication, inducing expectoratin, anodyne, relaxing the muscular spasm, even anticarcinogen. The liquorice root is indicated for weakness of the spleen and the stomach, lassitude, heart palpitation, short breath, cough, abundant expectoration, epigastric sag, spasm and pain of limbs, carbuncle, and sore. The liquorice root could alleviate the toxicity and the drastic actions of drug.

#### SUMMARY OF THE PRESENT INVENTION

An object of the present invention is to provide a pharmaceutical composition for strengthening spleen and stomach and a preparation method therefor.

The method provided by the present invention, comprises: mixing following four substances to obtain a mixture, i.e., the pharmaceutical composition for strengthening spleen and stomach, wherein the four substances are (A) ginseng ethanol extract, (B) volatile oil of largehead atractylodes rhizome and 5 tangerine peel, (C) aqueous extract of Indian bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome and rhizoma alismatis, and (D) fermentation broth of fruits and vegetables.

The substance (A) is prepared by a method comprising a 10 step of extracting an extract from ginseng with ethanol water by a reflux extraction to obtain the ginseng ethanol extract.

The substance (B) is prepared by a method comprising steps of soaking largehead atractylodes rhizome and tangerine peel in water, distilling the largehead atractylodes rhi- 15 zome and the tangerine peel to obtain distillate and dregs, and collecting the distillate to obtain the volatile oil of largehead atractylodes rhizome and tangerine peel.

The substance (C) is prepared by a method comprising steps of mixing Indian bread, vamaimo, lotus seed, liquorice 20 root, hawthorn fruit, germinated barley, rhizoma alismatis, the dregs obtained in preparation of the substance (B), and water, processing decoction and filtering, collecting filtrate, adding absolute ethyl alcohol, statically standing, and collecting clear solution to obtain the aqueous extract of Indian 25 meshes before the step of fermentation; bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome, and rhizoma alismatis.

The substance (D) is prepared by a method comprising steps of mixing fruits, vegetables, bacteria liquid of Lactoba- 30 cillus acidophilus, bacteria liquid of Bifidobactreium longum, bacteria liquid of Lactobacillus delbrueckii subsp. bulgaricus, and bacteria liquid of Streptococcus thermophilus, processing fermentation to obtain a fermentation product, i.e., the fermentation broth of fruits and vegetables.

A mass ratio of the ginseng, the largehead atractylodes rhizome, the Indian bread, the liquorice root, the tangerine peel, the yamaimo, the lotus seed, the hawthorn fruit, the germinated barley, and the rhizoma alismatis is (1.5~2.5):

The mass ratio of the ginseng, the largehead atractylodes rhizome, the Indian bread, the liquorice root, the tangerine peel, the yamaimo, the lotus seed, the hawthorn fruit, the germinated barley, and the rhizoma alismatis is preferably embodied as (1.5, 2 or 2.5):(1.5, 2 or 2.5):(1.5, 2 or 2.5):(0.5, 45) 1 or 1.5):(0.5, 1 or 1.5):(1.5, 2 or 2.5):(1.5, 2 or 2.5):(0.5, 1 or 1.5):(0.5, 1 or 1.5):(0.5, 1 or 1.5).

In a preparation method of the substance (A),

a period of the reflux extraction every time is 2 h~5 h, wherein the period of the reflux extraction is preferably 50 embodied as 2 h, 3 h or 5 h.

A concentration of the ethanol water is 70% (volume per-

In a preparation method of the substance (B),

a soaking period is (1~60) min, wherein the soaking period 55 is preferably embodied as 1 min, 30 min, or 60 min.

A distillating temperate is 90° C.~100° C., wherein the distillating temperature is preferably embodied as 90° C., 95° C., or 100° C.

In a preparation method of the substance (C),

a decoction temperature is 90° C.~100° C., wherein the decoction temperature is preferably embodied as 90° C., 95° C., or 100° C. A decoction period is 1 h~2 h, wherein the decoction period is preferably embodied as 1 h, or 2 h.

The step of adding the absolute ethyl alcohol comprises 65 adding the ethyl alcohol to the filtrate, until a volume percent of the ethyl alcohol in the filtrate is 70%.

In a preparation method of the substance (D),

a fermentation temperature is 18° C.~37° C., wherein the fermentation temperature is preferably embodied as 18° C., 23° C., or 37° C. A fermentation period is 10 days~180 days, wherein the fermentation period is preferably embodied as 10 days, 15 days, or 180 days. A fermentation method comprises stirring while fermentation.

The preparation method of the substance (A) further comprises steps of:

removing ethanol from the extract, and processing concentration and drying to obtain a dried product, i.e., the ginseng

The preparation method of substance (C) further comprises steps of removing ethyl alcohol from the clear solution after the step of collecting the clear solution, processing filtering to obtain filtrate, and concentrating and drying the filtrate to obtain a dried product, i.e., the aqueous extract of Indian bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome, and rhizoma alismatis.

The preparation method of the substance (D) further comprises steps of:

smashing the fruits and the vegetables into pieces of 40~50

after the step of fermentation, filtering the fermentation product, collecting filtrate, processing ultrafiltration, and collecting liquid produced by the ultrafiltration, i.e., the fermentation broth of fruits and vegetables.

The step of ultrafiltration comprises ultrafiltering the filtrate in a molecular weight of 100,000, wherein a liquid inlet pressure is 1.3 kg, and a liquid outlet pressure is 0.5 kg.

The fruits and the vegetables refer to a mixture of following 54 kinds of fruits and vegetables, which are

konjak, eggplant, asparagus, spinach, bean sprout, broccoli, cabbage, radish, cucumber, peas, red pepper, celery, scallion, garlic, grapes, grapefruit, watermelon, peach, tangerine, blue berry, sweet orange, banana, litchi, balsam pear, leek, pomegranate, pitaya, carrot, tomato, Chinese cabbage, (1.5~2.5):(1.5~2.5):(0.5~1.5):(0.5~1.5):(1.5~2.5):(1.5~2.5):(0.6~platiy)(49,5bell5)e(40pber, lleft)uce, pear, ginger, taro, kidney bean, pumpkin, lotus root, cherry, kiwi fruit, plum, strawberry, fig, kumquat, mandarin orange, Nanguo pear, cantaloup, Hami melon, papaya, onion, mulberry, sugar beet, and lemon.

Mass of each fruit or vegetable is equal with each other.

In the preparation method of the substance (D), every 1 L of the mixture is prepared by a step of mixing (5~50) g of the ginseng ethanol extract, (5~50) g of the volatile oil of largehead atractylodes rhizome and tangerine peel, (5~200) g of the aqueous extract of Indian bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome and rhizoma alismatis, and the fermentation broth of fruits and vegetables, wherein a volume of the mixture is up to 1 L by adding the fermentation broth of fruits and vegetables.

Every 1 L of the mixture is preferably prepared by a step of mixing (5, 25, or 50) g of the ginseng ethanol extract, (5, 30, or 50) g of the volatile oil of largehead atractylodes rhizome and tangerine peel, (5, 150, 200) g of the aqueous extract of Indian bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome and rhizoma alismatis, and the fermentation broth of fruits and vegetables, wherein a volume of the mixture is up to 1 L by adding the fermentation broth of fruits and vegetables.

In the preparation method of the substance (A), a proportion of the ethanol water and the ginseng is  $(1\sim10)$ ml: 1 g, wherein

the proportion of the ethanol water and the ginseng is preferably embodied as (1, 5, or 10) ml: 1 g.

In the preparation method of the substance (B),

a proportion of the largehead atractylodes rhizome, the tangerine peel and the water is  $(1\sim10)$  g: $(1\sim10)$  g: $(5\sim50)$  ml,

wherein the proportion of the largehead atractylodes rhizome, the tangerine peel and the water is preferably embodied as (1, 2, or 10) g:(1, 5, or 10) g:(5, 15, or 50) ml.

In the preparation method of the substance (C),

a proportion of the Indian bread, the yamaimo, the lotus seed, the liquorice root, the hawthorn fruit, the germinated barley, the rhizoma alismatis, and water is  $(1\sim10)$  g: $(1\sim10)$  $g:(1\sim10) g:(1\sim10) g:(1\sim10) g:(1\sim10) g:(1\sim10) g:(5\sim100) ml$ 

wherein the proportion of the Indian bread, the yamaimo, the lotus seed, the liquorice root, the hawthorn fruit, the germinated barley, the rhizoma alismatis, and water is preferably embodied as (1, 2, or 10) g:(1, 2, or 10) g:(1, 2, or 10) g:(1, 5, or 10) g:(1, 5, or 10) g:(1, 5, or 10) g:(1, 5, or 10) g:(50, or 10) g:(50,60, or 100) ml.

In the preparation method of the substance (D),

a proportion of the bacteria liquid of Lactobacillus acidophilus, the bacteria liquid of Bifidobactreium longum, the bacteria liquid of Lactobacillus delbrueckii subsp. bulgaricus, the bacteria liquid of Streptococcus thermophilus, the 25 fruits and vegetables, and water is (2000~8000) ml: (2000~8000) ml:(2000~8000) ml:(2000~8000) (1000~1500) kg:(1000~1500) kg,

wherein the proportion of the bacteria liquid of Lactobacillus acidophilus, the bacteria liquid of Bifidobactreium lon- 30 gum, the bacteria liquid of Lactobacillus delbrueckii subsp. bulgaricus, the bacteria liquid of Streptococcus thermophilus, the fruits and vegetables, and water is preferably embodied as (2000, 5000, or 8000) ml:(2000, 5000, or 8000) ml: (2000, 5000, or 8000) ml:(2000, 5000, or 8000) ml:(1000, 35 1200, or 1500) kg:(1000, 1200, or 1500) kg.

The bacteria liquid of Lactobacillus acidophilus is prepared by a method comprising: fermenting and cultivating Lactobacillus acidophilus to obtain a fermented product, i.e., the bacteria liquid of Lactobacillus acidophilus. A ferment- 40 ing temperature is 20° C.~41° C., wherein the fermenting temperature is preferably embodied as 20° C., 37° C., or 41° C. A fermenting period is 15 h~36 h, wherein the fermenting period is preferably embodied as 15 h, 16 h, or 36 h.

The bacteria liquid of *Bifidobactreium longum* is prepared 45 by a method comprising: fermenting and cultivating Bifidobactreium longum to obtain a fermented product, i.e., the bacteria liquid of Bifidobactreium longum. A fermenting temperature is 20° C.~41° C., wherein the fermenting temperature is preferably embodied as 20° C., 37° C., or 41° C. A 50 fermenting period is 15 h~36 h, wherein the fermenting period is preferably embodied as 15 h, 16 h, or 36 h.

The bacteria liquid of Lactobacillus delbrueckii subsp. bulgaricus is prepared by a method comprising steps of: fermenting and cultivating Lactobacillus delbrueckii subsp. 55 could all be obtained by a commercial way, if there is no bulgaricus to obtain a fermented product, i.e., the bacteria liquid of Lactobacillus delbrueckii subsp. bulgaricus. A fermenting temperature is 20° C.~41° C., wherein the fermenting temperature is preferably embodied as 20° C., 37° C., or 41° C. A fermenting period is 15 h~36 h, wherein the fer- 60 menting period is preferably embodied as 15 h, 16 h, or 36 h.

The bacteria liquid of Streptococcus thermophilus is prepared by a method comprising steps of: fermenting and cultivating Streptococcus thermophilus to obtain a fermented product, i.e., the bacteria liquid of Streptococcus thermophilus. A fermenting temperature is 20° C.~41° C., wherein the fermenting temperature is preferably embodied as 20° C., 37°

6

C., or 41° C. A fermenting period is 15 h~36 h, wherein the fermenting period is preferably embodied as 15 h, 16 h, or 36

The Lactobacillus acidophilus is preferably embodied as Lactobacillus acidophilus CGMCC 1.1854, the *Bifidobac*treium longum is preferably embodied as Bifidobactreium longum CGMCC 1.2186, the Lactobacillus delbrueckii subsp. bulgaricus is preferably embodied as Lactobacillus delbrueckii subsp. bulgaricus CGMCC 1.1480, and the Streptococcus thermophilus is preferably embodied as Streptococcus thermophilus CGMCC 1.2471.

A medium of fermenting and cultivating is prepared by a method comprising: mixing 10 g of peptone, 10 g of beef extract, 5 g of yeast extract, 20 g of glucose, 1 g of tween-80, 2 g of K<sub>2</sub>HPO<sub>4</sub>, 1 g of tween-80, 5 g of NaAC, 2 g of ammonium citrate tribasic, 0.2 g of MgSO<sub>4</sub>, 0.05 g of MnSO<sub>4</sub>, and water, wherein a volume of the medium is up to 1 L by adding the water. A medicine for reducing fatigue prepared by the method described as above is also in a protecting range of the present invention.

The largehead atractylodes rhizome is preferably embodied as parched largehead atractylodes rhizome, the lotus seed is preferably embodied as parched lotus seed, the hawthom fruit is preferably embodied as parched hawthom fruit, and the germinated barley is preferably embodied as parched germinated barley.

A medicine for strengthening spleen and stomach prepared is in the protecting range of the present invention.

Experiments in the present invention proves that the ginseng extract, the volatile oil of largehead atractylodes rhizome and tangerine peel, the aqueous extract of Indian bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome and rhizoma alismatis, enzyme of the fruits and vegetables (fermentation broth) extracted in the present invention have short extracting periods. The medicine for strengthening spleen and stomach could be prepared with the above raw materials. People with low immunity in China are mostly in sub-health, and associated with symptoms of high blood sugar. To solve this problem, seasonings in the present invention is preferably embodied as Baisheng sugar, in such a manner that a dosage of sweeteners tending to raise the blood sugar, such as glucose and sucrose, is reduced to benefit more people.

These and other objectives, features, and advantages of the present invention will become apparent from the following detailed description, the accompanying drawings, and the appended claims.

#### DETAILED DESCRIPTION OF THE PREFERRED **EMBODIMENT**

Experimental methods used in following embodiments are all conventional method, if there is no special instruction.

Material, reagents, etc. used in the following embodiments special instruction.

#### Example 1

Preparing Ginseng Enzyme Composition for Strengthening Spleen and Stomach

Method 1:

Raw materials of active constituent of the ginseng enzyme composition is listed as followed, which are 200 g of ginseng, 200 g of parched largehead atractylodes rhizome, 200 g of Indian bread, 100 g of liquorice root, 100 g of tangerine peel,

200 g of yamaimo, 200 g of parched lotus seed, 100 g of parched hawthom fruit, 100 g of parched germinated barley, and 100 g of rhizoma alismatis.

- I. Preparing Ginseng Extract Comprises:
- preparing the raw materials, wherein the ginseng (JiLin Zixin pharmaceutical co., LTD.) complies with regulations of <Pharmacopoeia of the Chinese People's Republic of China>, and qualities of the ginseng is strictly monitored according to enterprise standard requirements, before being fed;
- 2. weighing out the ginseng according to formula, processing washing and draining, processing reflux extraction twice with ethanol water having a concentration of 70% and a dosage 10 times (v/w) the dosage of the ginseng, wherein extracting period of every time is 3 h, processing filtering, combining filtrate, reducing pressure, and recycling ethanol; and
- 3. concentrating the filtrate until relative density of the filtrate is 1.10~1.15 (60° C. 0.07 MPa), and processing spray drying (inlet temperature is 170° C., and outlet temperature is 70° C.) to obtain spray drying powder, i.e., the ginseng extract.
- II. Preparing Volatile Oil of Largehead Atractylodes Rhizome and Tangerine Peel Comprises:
- 1. preparing the raw materials, wherein the largehead atractylodes rhizome and the tangerine peel (JiLin Zixin pharmaceutical co., LTD.) complies with regulations of <Pharmacopoeia of the Chinese People's Republic of China>, and qualities of the largehead atractylodes rhizome and the tangerine peel is strictly monitored according to enterprise standard requirements, before being fed; and
- 2. weighing out the largehead atractylodes rhizome and the tangerine peel according to the formula, processing washing and draining, soaking the largehead atractylodes rhizome and the tangerine peel in water for 30 min (proportion of the largehead atractylodes rhizome, the tangerine peel, and water is 2 g:15 ml), wherein dosage of the water added is preferably of which the water could just submerge medicinal materials, the dosage of the water is generally 5 times the dosage of the medicinal materials, and distilling temperature is 100° C., collecting the distillate with another container in a proportion of 5:1, (The volume collected is ½ the volume of the distillate, and the volatile oil

8

is extracted completely at this time) and collecting remaining medicine in a distilling container to obtain largehead atractylodes rhizome slag and tangerine peel slag.

III. Preparing Aqueous Extract of Indian Bread, Yamaimo, Lotus Seed, Tangerine Peel, Liquorice Root, Hawthorn Fruit, Germinated Barley, Largehead Atractylodes Rhizome and Rhizoma Alismatis Comprises:

- 1. preparing the raw materials, wherein the Indian bread, the yamaimo, the lotus seed, the liquorice root, the hawthorn fruit, the germinated barley, and the rhizoma alismatis, (JiLin Zixin pharmaceutical co., LTD.) complies with regulations of <Pharmacopoeia of the Chinese People's Republic of China>, and qualities of the Indian bread, the yamaimo, the lotus seed, the liquorice root, the hawthorn fruit, the germinated barley, and the rhizoma alismatis, is strictly monitored according to enterprise standard requirements, before being fed;
- weighing out the Indian bread, the yamaimo, the lotus seed, the liquorice root, the hawthorn fruit, the germinated barley, and the rhizoma alismatis according to the formula, and processing washing and draining; and
- 3. mixing the Indian bread, the yamaimo, the lotus seed, the liquorice root, the hawthorn fruit, the germinated barley, and the rhizoma alismatis with water, processing decoction twice, wherein decoction temperature is 100° C., dosages of the water are respectively 6 times and 5 times a dosage of medicinal material, periods are respectively 2 h and 1 h, combining filtrate of two times, concentrating the filtrate until relative density of the filtrate is 1.12~1.20 (80~90° C., reduced pressure concentration), adding ethanol until concentration of the ethanol is 70%, static standing for 24 h, collecting clear solution, recycling all of the ethanol, processing filtering, concentrating the filtrate until relative density of the filtrate is 1.10~1.15 (60° C., 0.07 MPa), and processing spray drying (inlet temperature is 170° C., and outlet temperature is 70° C.) to obtain spray drying powder, i.e., the aqueous extract, wherein in the first time of decoction, proportion of the Indian bread, the yamaimo, the lotus seed, the tangerine peel, the liquorice root, the hawthorn fruit, the germinated barley, the largehead atractylodes rhizome, the rhizoma alismatis, and water is 2 g:2 g:2 g:5 g:5 g:5 g:5 g:60 ml.

IV. Preparing Fermentation Broth of Fruits and Vegetables
1. The Fruits and Vegetables Selected

TABLE 1

	Fruits and vegetables					
Material	Nutritional ingredient	Material	Nutritional ingredient			
Konjac	Vitamin B1, B2, citric acid, fermentation product	Carrot	Vitamin A, carotene, fermentation product			
Eggplant	Vitamin A, B1, B2, C, fermentation product	Tomato	Vitamin A, carotene, citric acid, fermentation product			
Asparagus	Vitamin B1, B2, citric acid, fermentation product	Chinese cabbage	Vitamins, mineral substances, fermentation product			
Spinach	Vitamin A, C, ferrum, calcium, fermentation product	Parsley	Vitamins, mineral substances, fibers, fermentation product			
Bean sprout	Vitamin, saponin, amino acid, fermentation product	Bell pepper	Vitamin C, mineral substances, fermentation product			
Broccoli	Vitamin B1, B2, citric acid, fermentation product	Lettuce	Vitamin A, mineral substances, fermentation product			
Cabbage	Vitamin B1, B2, citric acid, fermentation product	Pear	Fructose, mineral substances, fermentation product			
Radish	Vitamin B1, B2, citric acid, fermentation product	Ginger	Vitamins, mineral substances, fermentation product			
Cucumber	Vitamin B1, B2, citric acid, fermentation product	Taro	Vitamin B1, B2, C, mineral substances, fermentation product			
Pea	Vitamin B1, B2, citric acid, fermentation product	Kidney beans	Vitamin B1, B2, citric acid, fermentation product			

	Fruits and vegetables					
Material	Nutritional ingredient	Material	Nutritional ingredient			
Red pepper	Vitamin B1, B2, citric acid, fermentation product	Pumpkin	Carotene, mineral substances, fermentation product			
Celery	Vitamin B1, B2, citric acid, fermentation product	Lotus root	Ferrum, tannin, fermentation			
Scallion	Vitamin B1, B2, citric acid, fermentation product	Cherry	mineral substances, fermentation product			
Garlic	Vitamin B1, B2, citric acid, fermentation product	Kiwi fruit	Vitamin C, fermentation product			
Grapes	Vitamin B1, B2, citric acid, fermentation product	Plum	organic acid, vitamins, fermentation product			
Grapefruit	Vitamin B1, B2, citric acid, fermentation product	Strawberry	Vitamin C, mineral substances, ellagic acid, fermentation product			
Watermelon	Vitamin B1, B2, citric acid, fermentation product	Fig	fermentation product, vitamins, mineral substances			
Peach	Vitamin B1, B2, citric acid, fermentation product	Kumquat	Vitamin B1, B2, C, fermentation product			
Tangerine	Vitamin B1, B2, C, fermentation product	Mandarin orange	Vitamin C, citric acid, fermentation product			
Blueberry	Vitamin B1, B2, citric acid, fermentation product	Nanguo pear	Vitamin B1, B2, C, citric acid, fermentation product			
Sweet orange	Vitamin B1, B2, citric acid, fermentation product	Cantaloup	Vitamin B1, B2, citric acid, fermentation product			
Banana	Vitamin B1, B2, citric acid, fermentation product	Hami melon	Fructose, potassium, vitamin A, fermentation product			
Litchi	Vitamin B1, B2, citric acid, fermentation product	Papaya	Vitamin B, C, E, citric acid, carotene, fermentation product			
Balsam pear	Vitamin B1, B2, citric acid, fermentation product	Onion	Vitamin B, C, carotene, fermentation product			
Leek	Vitamin B1, B2, citric acid, fermentation product	Mulberry	Vitamin B1, B2, citric acid, fermentation product			
Pomegranate	Vitamin B1, B2, citric acid, fermentation product	Sugar beet	Betaine, fermentation product			
Pitaya	Vitamin B1, B2, citric acid, fermentation product	Lemon	citric acid, fermentation product			

# 2. Fermenting the Fruits and the Vegetables

### 1) Selecting and Buying Strains

Probiotic strains are bought from micro-biology institute of Chinese Sciences Academy, the probiotics are Lactobacillus acidophilus CGMCC 1.1854, Bifidobactreium longum CGMCC 1.2186, Lactobacillus delbrueckii subsp. bulgaricus CGMCC 1.1480, and Streptococcus thermophilus CGMCC 1.2471. All of the strains are preserved in sand tube, and are used as original strains.

#### 2) Preparing Master Seeds

Preparing the master seeds of the probiotics (Generations of transfer of the mater seeds is not more than 10, and the generations in the present invention is 4) comprises:

(1) taking 1/10 of the sand tube of Lactobacillus acidophilus 50 CGMCC 1.1854, Bifidobactreium longum CGMCC 1.2186, Lactobacillus delbrueckii subsp. bulgaricus CGMCC 1.1480, and Streptococcus thermophilus CGMCC 1.2471 with a sterile stainless steel spoon, wherein rest of the probiotics is cryopreserved, respectively inoculating the probiotics 55 of: (The transfer generations of working seeds is not more in 50 ml of MRS liquid medium (250 triangular flask) (10 g/l peptone, 10 g/l beef extract, 5 g/l yeast extract, 20 g/l glucose, 1 g/l tween-80, 2 g/l K<sub>2</sub>HPO<sub>4</sub>, 1 g/l tween-80, 5 g/l NaAC, 2 g/l ammonium citrate tribasic, 0.2 g/l MgSO<sub>4</sub>, 0.05 g/l MnSO<sub>4</sub>, sterilization in a temperature of 121° C. for 20 min), and 60 culturing the probiotics on a shaking table in a speed of 100 r/min and a temperature of 37° C. for 16 h;

(2) respectively taking one loop of each probiotics with a inoculating loop, respectively streak inoculating the probiotics in MRS solid medium (10 g/l peptone, 10 g/l beef extract, 65 5 g/l yeast extract, 20 g/l glucose, 1 g/l tween-80, 2 g/l K<sub>2</sub>HPO<sub>4</sub>, 1 g/l tween-80, 5 g/l NaAC, 2 g/l ammonium citrate

tribasic, 0.2 g/l MgSO<sub>4</sub>, 0.05 g/l MnSO<sub>4</sub>, 1.5% agar, sterilization in a temperature of 121° C. for 20 min), culturing the probiotics in a incubator in a temperature of 37° C. for 16 h;

(3) respectively taking a bacterial colony which is most eugenic in each probiotics, respectively inoculating the bacterial colony in 50 ml of the MRS liquid medium, and culturing the bacterial colony on the shaking table in a speed of 180 r/min and a temperature of 30° C. for 16 h;

(4) respectively inoculating the bacterial colony in 500 ml 45 of the MRS liquid medium, culturing the bacterial colony on the shaking table in a speed of 100 r/min and a temperature of 37° C. for 16 h, adding glycerol to a concentration of 20%, shaking up, storing the probiotics by 1 ml into freezing tubes, which are used as the master seeds of Lactobacillus acidophilus, Bifidobactreium longum, Bifidobacterium breve, and Streptococcus thermophilus, preserving in a temperature of -40° C.

#### 3. Preparing Working Seeds

Preparing working seeds of the probiotics comprises steps than 5, and the transfer generations in the present invention is

- (1) respectively taking the master seeds of Lactobacillus acidophilus CGMCC 1.1854, Bifidobactreium longum CGMCC 1.2186, Lactobacillus delbrueckii subsp. bulgaricus CGMCC 1.1480, and Streptococcus thermophilus CGMCC 1.2471 with the sterile inoculating loop, respectively streak inoculating the master seeds on the MRS solid medium, and culturing the master seeds in the incubator in a temperature of 37° C. for 16 h;
- (2) respectively taking a bacterial colony which is most eugenic in each probiotics, respectively inoculating the bac-

terial colony in 50 ml of the MRS liquid medium, and culturing the bacterial colony on the shaking table in a speed of 100 r/min and a temperature of  $37^{\circ}$  C. for 16 h;

- (3) respectively inoculating the bacterial colony in 500 ml of the MRS liquid medium, and culturing the bacterial colony on the shaking table in a speed of  $100 \, \text{r/min}$  and a temperature of  $37^{\circ}$  C. for  $16 \, \text{h}$ ; and
- (4) respectively inoculating the bacterial colony in 5000 ml of the MRS liquid medium, and culturing the bacterial colony on the shaking table in a speed of 100 r/min and a temperature of 37° C. for 16 h to obtain bacteria liquid of *Lactobacillus acidophilus* CGMCC 1.1854, bacteria liquid of *Bifidobactreium longum* CGMCC 1.2186, bacteria liquid of *Lactobacillus delbrueckii* subsp. *bulgaricus* CGMCC 1.1480, and bacteria liquid of *Streptococcus thermophilus* CGMCC 1.2471, wherein above bacteria liquids are all whole fermenting products in the fermenting container.
- 4. Treating the Fruits and the Vegetables, Comprises Steps of:
- 1) Weighing the above materials;
- washing the above materials, removing foreign substances, drying, and weighing; and
- 3) smashing the materials into pieces of 40~50 meshes, adding the materials into a fermenter; wherein practical feeding amount is 2400 kg for a fermenter of 3 t (Weight of effective solvents=3 t\*0.8=2.4 t), and a proportion of the fruits and vegetables and water is 1:1, e.g. the weight of the fruits and vegetables is 1200 kg, and the weight of the water is 1200 kg.
- 5. Fermenting the Fruits and the Vegetables with Rapid Zymolyte, Comprises Steps of:
- Adding 5000 ml of each of the bacteria liquid of Lactobacillus acidophilus CGMCC 1.1854, the bacteria liquid of Bifidobactreium longum CGMCC 1.2186, the bacteria liquid of Lactobacillus delbrueckii subsp. bulgaricus CGMCC 1.1480, and the bacteria liquid of Streptococcus thermophilus CGMCC 1.2471 into the fermenter, controlling the fermenting temperature being 23° C., stirring for 15 d:
- filtering the fermenting product with a filter cloth of 200 meshes, and removing residues of the fruits and vegetables to obtain filtrate;
- 4) ultrafiltering the filtrate in a molecular weight of 100,000 (a liquid inlet pressure is 1.3 kg, and a liquid outlet pressure is 0.5 kg) to obtain 1200~1500 kg of clear liquid, sealing the clear liquid, preserving the clear liquid in a temperature of 4° C. to obtain the fermentation broth of fruits and vegetables.

# 6. Detecting

The fermentation broth of fruits and vegetables mainly comprises: lactic acid and acetic acid, so acidity is identified as characteristic components of the fermentation broth of fruits and vegetables, which is preferably embodied as followed

The acidity of the fermentation broth of fruits and vegetables refers to ml number/volume of NaOH solution of 0.1N consumed to titrate 100 ml of the fermentation broth of fruits and vegetables, and 10 ml of sample of the fermentation broth of fruits and vegetables is usually used when detecting.

Detecting comprises steps of: taking 10 ml of the fermentation broth of fruits and vegetables, 20 ml of water, and 0.5 ml of phenolphthalein indicator, processing titration with 65 NaOH standard solution of 0.1N until mixture turns to reddish without fading in a period of 30 seconds. Calculating

12

formula: Acidity=volume of the NaOH standard solution of 0.1N consumed\*10.

Result: The acidity of the product is 42.

#### V. Preparing the Composition

The exacts and the fermentation broth of fruits and vegetables are mixed according formula as followed. 25 g of the ginseng ethanol extract, 30 g of the volatile oil of largehead atractylodes rhizome and tangerine peel, 150 g of the aqueous extract of Indian bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome and rhizoma alismatis, and the fermentation broth of fruits and vegetables are mixed to obtain the composition, wherein the fermentation broth of fruits and vegetables supplements volume of the mixture to 10,000 ml, and the 10,000 ml of the mixture could be divided into 1000 bottles, 10 ml per bottle.

Method 2:

Raw materials of active constituent of the ginseng enzyme composition is listed as followed, which are 150 g of ginseng, 150 g of parched largehead atractylodes rhizome, 150 g of Indian bread, 50 g of liquorice root, 50 g of tangerine peel, 150 g of yamaimo, 150 g of parched lotus seed, 50 g of parched hawthom fruit, 50 g of parched germinated barley, and 50 g of rhizoma alismatis.

I. Preparing Ginseng Extract Comprises Steps of:

Extracting method is mainly same as method 1, except that the ginseng refluxing extracted with ethanol water having a concentration of 70% and a dosage 1 times (v/w) the dosage of the ginseng, wherein extracting period of every time is 2 h. II. Preparing Volatile Oil of Largehead Atractylodes Rhizome and Tangerine Peel

Extracting method is mainly same as method 1, except that the proportion of the largehead attractylodes rhizome, the tangerine peel, and water is 1 g:5 g:5 ml, soaking period is 1 min, and the distilling temperature is 90° C.

III. Preparing Aqueous Extract of Indian Bread, Yamaimo, Lotus Seed, Tangerine Peel, Liquorice Root, Hawthorn Fruit, Germinated Barley, Largehead Atractylodes Rhizome and Rhizoma Alismatis

IV. Preparing Fermentation Broth of Fruits and Vegetables
Extracting method is mainly same as method 1, except th

Extracting method is mainly same as method 1, except that 2000 ml of each of the bacteria liquid of *Lactobacillus acidophilus* CGMCC 1.1854, the bacteria liquid of *Bifidobactreium longum* CGMCC 1.2186, the bacteria liquid of *Lactobacillus delbrueckii* subsp. *bulgaricus* CGMCC 1.1480, and the bacteria liquid of *Streptococcus thermophilus* CGMCC 1.2471 are added, the weight of the fruits and vegetables added is 1000 kg, and the weight of the water is 1000 kg.

Fermenting temperature of the fermentation broth of fruits and vegetables is 18° C., and fermenting period is 10 d.

In the preparation of the bacteria liquid of *Lactobacillus acidophilus*, the bacteria liquid of *Bifidobactreium longum*, the bacteria liquid of *Lactobacillus delbrueckii* subsp. *bulgaricus*, and the bacteria liquid of *Streptococcus thermophilus*, fermenting temperatures are all 20° C., and fermenting periods are all 15 h.

Detecting method is same as method 1, and there is no marked difference in the result and the steps.

#### V. Preparing the Composition

The preparing method is mainly same as method 1, except that 5 g of the ginseng ethanol extract, 5 g of the volatile oil of largehead atractylodes rhizome and tangerine peel, 5 g of the aqueous extract of Indian bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome and rhizoma alismatis, and

the fermentation broth of fruits and vegetables are mixed to obtain the composition, wherein the fermentation broth of fruits and vegetables supplements volume of the mixture to 10 000 ml

Method 3:

Raw materials of active constituent of the ginseng enzyme composition is listed as followed, which are 250 g of ginseng, 250 g of parched largehead atractylodes rhizome, 250 g of Indian bread, 150 g of liquorice root, 150 g of tangerine peel, 250 g of yamaimo, 250 g of parched lotus seed, 150 g of parched hawthom fruit, 150 g of parched germinated barley, and 150 g of rhizoma alismatis.

I. Preparing Ginseng Extract Comprises Steps of:

Extracting method is mainly same as method 1, except that the ginseng refluxing extracted with ethanol water having a concentration of 70% and a dosage 5 times (v/w) the dosage 15 of the ginseng, wherein extracting period of every time is 5 h. II. Preparing Volatile Oil of Largehead Atractylodes Rhizome and Tangerine Peel

Extracting method is mainly same as method 1, except that the proportion of the largehead atractylodes rhizome, the tangerine peel, and water is  $10\,\mathrm{g}{:}10\,\mathrm{g}{:}50\,\mathrm{ml}$ , soaking period is 60 min, and the distilling temperature is  $95^{\circ}$  C.

III. Preparing Aqueous Extract of Indian Bread, Yamaimo, Lotus Seed, Tangerine Peel, Liquorice Root, Hawthorn Fruit, Germinated Barley, Largehead Atractylodes Rhizome and Rhizoma Alismatis

Extracting method is mainly same as method 1, except that 8000 ml of each of the bacteria liquid of *Lactobacillus acidophilus* CGMCC 1.1854, the bacteria liquid of *Bifidobactreium longum* CGMCC 1.2186, the bacteria liquid of *Lactobacillus delbrueckii* subsp. *bulgaricus* CGMCC 1.1480, and the bacteria liquid of *Streptococcus thermophilus* CGMCC 1.2471 are added, the weight of the fruits and vegetables is 1500 kg, and the weight of the water is 1500 kg.

Fermenting temperature of the fermentation broth of fruits and vegetables is 37, and fermenting period is 180 d.

In the preparation of the bacteria liquid of *Lactobacillus acidophilus*, the bacteria liquid of *Bifidobactreium longum*, the bacteria liquid of *Lactobacillus delbrueckii* subsp. *bulgaricus*, and the bacteria liquid of *Streptococcus thermophilus*, fermenting temperature are all 41° C., and fermenting period is 36 h.

Detecting method is same as method 1, and there is no marked difference in the result and the steps.

V. Preparing the Composition

The preparing method is mainly same as method 1, except that 50 g of the ginseng ethanol extract, 50 g of the volatile oil of largehead atractylodes rhizome and tangerine peel, 200 g of the aqueous extract of Indian bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome and rhizoma alismatis, and the fermentation broth of fruits and vegetables are mixed to obtain the composition, wherein the fermentation broth of fruits and vegetables supplements volume of the mixture to 10.000 ml.

### Example 2

Preparation of Oral Solution of Ginseng, Largehead Atractylodes Rhizome, and Indian Bread

A conventional oral solution producing process is used to producing this product, and the producing process comprises 14

steps of: mixing, filtering, instantaneous sterilization, bottling, capping, leakage detecting, checking transparent degree, outer packing, inspection, and entering warehouse, wherein the steps before leakage detecting are all processed in 100,000 class clean area. Producing process design and equipment conditions both conform to GMP requirement of China.

Specific producing method comprises steps of mixing the composition obtained in example 1 and seasoning (The seasoning is white granulated sugar, bought from Eurasia department of Changehun.), adding mixture into a liquid tank, stirring for 30 min to ensure uniformity, and instantaneous high temperature sterilization to obtain oral solution of ginseng, largehead atractylodes rhizome, and Indian bread.

- 2. Bottling the product into oral solution bottles comprises step of: bottling the product into oral solution bottles with an oral solution bottling machine, wherein every bottle is 10 ml, packing volume difference of product should be monitored while bottling to ensure that the difference is controlled in a difference range.
- 3. Capping comprises steps of: capping the oral solution with an oral solution capping machine, and screening non-conforming products.
- 4. Leakage detecting comprises a step of: detecting leakage of the oral solution capped with vacuum leakage detector.
- 5. Inner packing comprises a step of: packing with cardboard boxes, wherein every cardboard box comprises 10 bottles
- 6. Outer packing comprises a step of: packing with corrugated cases.
- 7. Detecting comprises a step of detecting according to a enterprise standard.
- 8. Entering warehouse comprises a step of displacing the product into a warehouse after the product is inspected to be qualified.

#### Example 3

Experimental Research on how the Oral Solution of Ginseng, Largehead Atractylodes Rhizome, and Indian Bread Improves Digestion

1. Materials

1.1. Experimental Animals

60 mice of clean level and Kunming species weighing 18~22 g and 20 male rats of clean level and Wistar weighing 180~200 g are provided by animal experimental center of Jilin university.

1.2. Experimental Medicine

1.2.1. Medicine

The oral solution of ginseng, largehead atractylodes rhizome, and Indian bread is prepared in example 2, and Motilium tablets is produced by Xian-Janssen Pharmaceutical Ltd and has a specification of 100mg\*30 pieces.

1.2.2. Preparing of Medicine

Motilium: 10mg of Motilium is added into 100m1 of distilled water to obtain Motilium solution, wherein each milliliter of the Motilium solution contains 0.1mg of Motilium. 1.3. Main Agentia

Paraformaldehyde (E. Merk); Acetylthiocholine iodide (Fluka); Dextran blue 2000 (pharmacia); Sodium acetate (Shanghai chemical reagent station); Citron acid sodium (Qingdao marine chemical plant); Copper sulfate (Shenyang Hongvitality chemical plant); Potassium ferricyanide (Taizhou biochemical materials plant); and Four isopropyl phosphorous amides (Qingdao Marine chemical plant).

1.4. Main Apparatus

Cryostat frozen section machine; Desktop balance recorder; Full wavelength spectrophotometer, centrifugal

sink lake; Optical microscope; Multimedia color pathological image analysis system, and OlympusPM-30 photographic system.

#### 2. Method

All of counting data and measurement data are expressed 5 as mean±standard deviation (x±s). Compare between two groups is tested by U/t, and analysis of variance (ANOVA) is used to compare multiple groups. The data are processed by medicine SAS statistical software package on the computer. 2.1. Detecting Advancing Function of Small Intestine of a 10 Mouse

30 mice of Kunming species are taken, which have fasted for 20~24h. The mice are randomly divided into 3 groups, i.e., a control group, a group of oral solution of ginseng, largehead atractylodes rhizome, and Indian bread, and a Motilium 15 group. Each group comprises 10 animals, tagged with picric acid. The group of oral solution of ginseng, largehead atractylodes rhizome and Indian bread are processed with intragastric administration by feeding stoste of the oral solution of ginseng, largehead atractylodes rhizome and Indian bread 20 (20.0ml/kg). The Motilium group is processed with intragastric administration by feeding the Motilium solution (3.9mg/ kg). The control group is processed with intragastric administration by feeding equivalent amount of physiological saline. After 90 minutes, each group is given 0.3ml of ink for 25 intragastric administration. After 20 minutes, the mice are executed by cervical dislocation. For each mouse, an abdominal cavity is opened, a mesentery is separated, a length of intestinal canal is cut off from pylorus in an upper portion to ileocecus in a lower portion, and the length of intestinal canal 30 is displaced on a tray. A small intestine is gently pulled into a straight line, and the length of the intestinal canal is measured as a total length of the small intestine. A distance between the pylorus and a front edge is defined as "advancing distance of the ink in the small intestine". An advancing rate of the ink is 35 calculated by a formula.

Advancing rate of the ink(%)=advancing distance of the ink in the small intestine (cm)/the total length of the small intestine (cm)\*100%

Comparing results of the advancing function of small intestine of the three groups is illustrated in Table 2.

16

Compared with the control group,  $\Delta P$ <0.01. Compared with the Motilium group, \*P<0.05.

# 2.2. Detecting Gastric Emptying Function of a Mouse

30 mice are divided into a group of oral solution of ginseng, largehead atractylodes rhizome, and Indian bread, Motilium group, and a control group. Each group comprises 10 mice. The group of oral solution of ginseng largehead atractylodes rhizome and Indian bread is processed with intragastric administration by feeding the oral solution of ginseng largehead atractylodes rhizome and Indian bread of 20.0ml/kg. The Motilium group is processed with intragastric administration by feeding the Motilium solution of 3.9mg/kg. The control group is processed with intragastric administration by feeding equivalent volume of deionized water. After 10 minutes, 0.4ml of the dextran blue 2000 solution of 2% is given to each mouse. After 30 minutes, the mice are executed by cervical dislocation. For each mouse, all of stomach and intestines are taken out after opening abdominal cavity, and the stomach is cut off at a position of pylorus sphincter. Residual pigment (dextran blue 2000) in the stomach is fully dissolved in 2ml of deionized water, and solution containing pigment is centrifugally separated in 3500rpm for 15 minutes. Then supernatant is taken and filtered by glass cotton filter. Absorbance of filtrate is measured by giving a light of 620nm with a 723 type spectrophotometer, to obtain a stomach pigment residual quantity and calculate mean value of each group. The mean value of the control group is defined as 100%, and percentage of the mean values of other groups and the mean value of the control group is defined as relative stomach pigment residual rate.

TABLE 3

Comparison of stomach pigment residual rate of three groups $(x \pm s)$						
Experimental group	Amount of animals	Somach pigment residual quantity	Rlative stomach pigment residual rate (%)			
Control group Ginseng largehead atractylodes	10 10	186.6 ± 42.6 94.7 ± 23.6Δ*	100 50.9 <b>Δ*</b>			

TABLE 2

Comparison of	of advancing	function of	small in	itestine of t	the three	groups (	$(x \pm s)$

Experimental group	Amount of animals	Total length of small intestine (cm)	Advancing distance of ink in small intestine (cm)	Advancing rate(%)
Control group	10	41.02 ± 2.7959	26.450 ± 4.0445	64.27 ± 7.0015
Ginseng	10	37.82 ± 3.3133	31.38 ± 4.2923Δ*	83.017 ± 8.7961
largehead				
atractylodes				
rhizome and				
Indian bread				
oral solution				
group				
Motilium group	10	37.82 ± 5.2087	$27.0 \pm 2.7406$	71.938 ± 6.8437

18 TABLE 4

Comparison of stomach pigment residual rate of three groups (x ± s)				_	Comparison of cont	attraction amplitude of four groups of rats $(x \pm s)$		
	anee	у длоцро (н = 5)	Distance of the state of the st	— <sub>5</sub>	Experimental group	Amount of animals	Contraction amplitude	
Experimental group	Amount of animals	Somach pigment residual quantity	Rlative stomach pigment residual rate (%)	,	Control group Second model group	5 5	18.72 ± 0.8818 14.976 ± 0.9465	
rhizome and Indian bread oral solution group Motilium group	10	152.3 ± 14.01	81.8	10	Group of oral solution of ginseng, largehead atractylodes rhizome, and Indian bread Motilium group	5	21.024 ± 1.7972*▲  17.372 ± 1.6270*	
Compared with the con			01.0	_	Compared with the control g	roup, ΔP < 0.01.	17.372 1 1.0270	

Compared with the control group,  $\Delta P < 0.01$ . Compared with the Motilium group, \*P < 0.01.

#### 2.3. Establishment of Animal Model

A characteristics of the rats is used according to a method of enraging stimulation. The characteristics of the rats is that the rats are grumpy and easy to be enraged, so anintense fighting between the rats could be caused by a low intensity stimulation. 20 male rats of clean level and Wistar provided by animal experimental center of Jilin university are randomly divided into 4 groups, i.e., three first model group and a control group. For the first model groups, tails of the rats are stimulated by being clipped with a forceps hamostatic, which 25 is wrapped by a gauze in a top end, in such a manner that the rats are enraged, and fight with each other. Stimulation is processed for 30 minutes every time, every 3 hours once, and 4 times a day. After a week, intake of food and drink significantly decreases, and some rats even don't eat and drink at all. 30 The rats are mainly tired. The hair of the rats becomes pale, withered, and yellow. Consequently, weights of the rats decrease. The rats in the first model groups present typical symptoms of disharmony between liver and stomach, and non-ulcer dyspepsia (NUD).

# 2.4. Detecting Gastric Motility Frequency and Amplitude of

The rats in the first model groups, which present symptoms of disharmony between liver and stomach, are randomly 40 divided into a second model group, a group of oral solution of ginseng, largehead atractylodes rhizome, and Indian bread, and a group of Motilium group. Each group comprises 5 rats. The group of oral solution of ginseng, largehead atractylodes rhizome, and Indian bread are treated with intragastric administration by feeding the oral solution of ginseng, largehead atractylodes rhizome, and Indian bread of 20.0ml/kg·d at twice. The Motilium group is treated with intragastric administration by feeding the Motilium solution of 2.7mg/kg.d at 500 three times. The second model group is processed with intragastric administration by feeding equivalent amount of physiological saline. Each of the groups has a courses of 2 weeks. After the treatment is finished, the animal is anesthetized with urethane of 10%, and then fixed on a operating table in a 55 supine position. An abdominal cavity of the rat is cut from a middle of an abdominal wall. A longitudinal muscle is found near a gastric antrumin in a greater gastric curvature. A near end of the longitudinal muscle is sutured by 1 needle to being fixed. A far end of the longitudinal muscle is sutured by 1 60 needle. A first end of a string is connected with the far end, and a second end of the string is connected with a tonotransducer. Motility frequencies and motility amplitudes of stomach begin to be recorded by a desktop balance recorder at 1h after operations. An initial tension is set to 5mv. None of the 65 experimental animals presents organic diseases such as ulcer, which is also observed from the experiments.

TABLE 5

	Comparison of contraction frequency of four groups of rats $(x \pm s)$					
)	Experimental group	Amount of animals	Contraction frequency (times/minutes)			
	Control group	5	3.92 ± 0.1095			
	Second model group	5	$2.526 \pm 0.4604\Delta$			
5	Group of oral solution of ginseng, largehead atractylodes rhizome, and Indian bread	5	4.364 ± 0.4336*			
	Motilium group	5	$3.82 \pm 0.2864$			

Compared with the control group,  $\Delta P < 0.01$ . Compared with second model group, \*P < 0.01. Compared with the Motilium group, ▲P < 0.05.

Compared with second model group, \*P < 0.01.

Compared with the Motilium group, ▲P < 0.05.

It is shown by experimental results that the rats in the second model group don't present organic diseases, such as ulcer, but the mechanical contraction frequency and the 35 mechanical contraction amplitude of the stomach and the intestines of the second model group are significantly poorer than those of the control group, which proves that negative emotions, such as tension, anxiety, irritability, are important factors causing the non-ulcer dyspepsia.

It is improved by research that the oral solution of ginseng, largehead atractylodes rhizome, and Indian bread could recover normal mechanical motion of the stomach efficiently. After the model animals is treated by the present composition, the contraction frequency and the contraction amplitude of the stomach both increase significantly. The composition could also significantly improve the gastric emptying function and the advancing function of small intestine. In summary, the oral solution of ginseng, largehead atractylodes rhizome, and Indian bread could treat the non-ulcer dyspepsia

The compositions prepared by the method 2 and the method 3 of the example 1 are detected by a method same as above, and results are also same as the composition 1 prepared by the method 1.

One skilled in the art will understand that the embodiment of the present invention as shown in the drawings and described above is exemplary only and not intended to be

It will thus be seen that the objects of the present invention have been fully and effectively accomplished. Its embodiments have been shown and described for the purposes of illustrating the functional and structural principles of the present invention and is subject to change without departure from such principles. Therefore, this invention includes all modifications encompassed within the spirit and scope of the following claims.

What is claimed is:

1. A method for preparing a pharmaceutical composition for strengthening spleen and stomach, comprising steps of:

preparing a substance (A), wherein the substance (A) is ginseng ethanol extracts, prepared by the steps of 5 extracting extracts from ginseng with ethanol water by a reflux extraction, removing ethanol from the extracts, concentrating and drying to obtain the ginseng ethanol extracts:

preparing a substance (B), wherein the substance (B) is volatile oils of largehead atractylodes rhizome and tangerine peel, prepared by the steps of soaking largehead atractylodes rhizome and tangerine peel in water, distilling the largehead atractylodes rhizome and the tangerine peel to obtain distillate and dregs, and collecting the distillate to obtain the volatile oils of largehead atractylodes rhizome and tangerine peel;

preparing a substance (C), wherein the substance (C) is extracts of Indian bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, 20 largehead atractylodes rhizome and rhizoma alismatis, prepared by the steps of mixing Indian bread, yamaimo, lotus seed, liquorice root, hawthorn fruit, germinated barley, rhizoma alismatis, the dregs obtained in preparation of the substance (B), and water, making a decoc- 25 tion and filtering, collecting filtrate, adding absolute ethyl alcohol, letting it stand in order to decant, collecting the clear solution, removing ethyl alcohol from the clear solution after the step of collecting the clear solution, filtering the solution to obtain a filtrate and concentrating and drying the filtrate to obtain the aqueous solution extracts of Indian bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome and rhizoma alismatis;

preparing a substance (D), wherein the substance (D) is fermentation broth of fruits and vegetables, prepared by the steps in order of mixing fruits, vegetables, bacteria liquid of *Lactobacillus acidophilus*, bacteria liquid of *Bifidobacterium longum*, bacteria liquid of *Lactobacillus delbrueckii* subsp. *bulgaricus*, and bacteria liquid of *Streptococcus thermophilus*, smashing the fruits and the vegetables into pieces with a size of 40-50 meshes, fermenting the mixture filtering the fermentation product, collecting the filtrate, ultrafiltering, and collecting the 45 fermentation broth of fruits and vegetables produced by the ultrafiltration; and

mixing the substances (A), (B), (C) and (D) to obtain the pharmaceutical composition for strengthening spleen and stomach.

2. A method for preparing a pharmaceutical composition for strengthening spleen and stomach, comprising steps of: preparing a substance (A), wherein the substance (A) is ginseng ethanol extracts, prepared by the a step of

20

extracting extracts from ginseng with ethanol water by a reflux extraction to obtain the ginseng ethanol extracts; preparing a substance (B), wherein the substance (B) is volatile oils of largehead atractylodes rhizome and tangerine peel, prepared by the steps of soaking largehead atractylodes rhizome and tangerine peel in water, distilling the largehead atractylodes rhizome and the tangerine peel to obtain distillate and dregs, and collecting the distillate to obtain the volatile oils of largehead atractylodes rhizome and tangerine peel;

preparing a substance (C), wherein the substance (C) is extracts of Indian bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome and rhizoma alismatis, prepared by the steps of mixing Indian bread, yamaimo, lotus seed, liquorice root, hawthorn fruit, germinated barley, rhizoma alismatis, the dregs obtained in preparation of the substance (B), and water, making a decoction and filtering, collecting filtrate, adding absolute ethyl alcohol, letting it stand in order to decant, and collecting the clear solution to obtain the aqueous solution extracts of Indian bread, yamaimo, lotus seed, tangerine peel, liquorice root, hawthorn fruit, germinated barley, largehead atractylodes rhizome and rhizoma alismatis;

preparing a substance (D), wherein the substance (D) is fermentation broth of fruits and vegetables, prepared by the steps in order of mixing fruits, vegetables, bacteria liquid of *Lactobacillus acidophilus*, bacteria liquid of *Bifidobacterium longum*, bacteria liquid of *Lactobacillus delbrueckii* subsp. *bulgaricus*, and bacteria liquid of *Streptococcus thermophilus*, and fermenting the mixture to obtain the fermentation broth of fruits and vegetables; and

mixing the substances (A), (B), (C) and (D) to obtain the pharmaceutical composition for strengthening spleen and stomach.

wherein the fruits and vegetables under (D) are a mixture of the following 54 kinds of fruits and vegetables:

konjak, eggplant, asparagus, spinach, bean sprout, broccoli, cabbage, radish, cucumber, peas, red pepper, celery, scallion, garlic, grapes, grapefruit, watermelon, peach, tangerine, blueberry, sweet orange, banana, litchi nut, balsam pear, leek, pomegranate, pitaya, carrot, tomato, Chinese cabbage, parsley, bell pepper, lettuce, pear, ginger, taro, kidney bean, pumpkin, lotus root, cherry, kiwi fruit, plum, strawberry, fig, kumquat, mandarin orange, Nanguo pear, cantaloupe, Hami melon, papaya, onion, mulberry, sugar beet, and lemon, wherein the

mass of each fruit or vegetable is equal with each other.

\* \* \* \* \*